EFFECTS OF TRANSMEMBRANE PRESSURE (TMP) ON THE PERFORMANCE OF ULTRAFILTRATION (UF) MEMBRANE OF SOY PROTEIN

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I declare that this thesis entitled "Effects of Transmembrane Pressure (TMP) on the Performance of Ultrafiltration (UF) Membrane of Soy Protein" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature :………………………………

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Date : 30 April 2009

To my family members,
my friends – reality or virtual,
my fellow colleagues,
and all faculty members

And for those who keep whispering…
"You can do it!!!"

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ABSTRACT

The use of membrane separations using ultrafiltration (UF) in the fractionation of soy protein from soy milk has generated considerable interest, recently. Compared to traditional methods, UF gives higher yield and superior functional properties of the soy protein, and also allows the recovery of active soybean trypsin inhibitor (STI) for medical purposes. The performance of the UF membrane; in term of permeate flux, concentration of protein transmitted and retention ratio, by manipulating transmembrane pressure (TMP) to fractionate soy protein from soy milk is studied. Commercial soy milk samples have been centrifuged and introduced to the UF system at various TMP value. Data from the permeate flux, concentration of protein transmitted and retention ratio have been manipulated to get idea on the performance of the UF membrane. Based on the results, the range of effective TMP to fractionate soy protein from soy milk is between 15 to 20 psi. In this TMP range, the permeate flux is between 25 and 30 LMH, the concentration of protein transmitted is between 1.38 and 2.85 mg/mL and the retention ratio is 90 percent.

ABSTRAK

Proses pemisahan membran menggunakan ultraturasan (UF) dalam memisahkan protein soya dari susu soya semakin mendapat perhatian lewat kebelakangan ini. Berbanding dengan cara tradisional, UF memberikan hasil yang banyak dan ciri berfungsi protein soya yang tinggi, di samping membenarkan perencat tripsin kacang soya (STI) untuk dikumpulkan. Prestasi membran UF dengan memanipulasikan tekanan antara membran (TMP) untuk memisahkan protein soya dari susu soya melalui fluks; kepekatan protein menembusi membran, dan nisbah penolakan membran, dikaji. Susu soya komersil terempar dilalukan pada sistem UF pada beberapa nilai TMP yang ditetapkan. Maklumat dari fluks; kepekatan protein menembusi membran, dan nisbah penolakan membran telah dimanipulasikan untuk mendapatkan gambaran mengenai prestasi membran UF. Berdasarkan keputusan, julat efektif TMP adalah antara 15 dan 20 psi. Dalam julat tersebut, nilai fluks adalah antara 25 dan 30 LMH, kepekatan protein menembusi membran adalah antara 1.38 dan 2.85 mg/mL dan nisbah penolakan membran adalah 90 peratus.

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LIST OF ABBREVIATIONS

BSA	- Bovine serum albumin
DI water	- Deionized water
kDa	- kiloDalton
M	- Molar
MWCO	- Molecular Weight Cut-Off
NaOH	- Sodium Hydroxide
OD	- Optical Density
PES	- Polyethersulfone
S	- Sieving coefficient
STI	- Soybean Trypsin Inhibitor
TMP	- Transmembrane pressure
UF	- Ultrafiltration

LIST OF SYMBOLS

%	- Percent
°C	- Degree Celcius
cm / s	- Centimeter per second
cm / s. kPa	- Centimeter per second kiloPascal
ft^2	- Feet squared
g	- Gram
g / mL.	- Gram per milliliter
kPa	- KiloPascal
L	- Liter
lb / in	- Pound per inch
LMH	- Liter per meter squared per hour
m^2	- Meter squared
m^3	- Meter cubic
mg / L	- Milligram per liter
mL	- Milliliter
m / s	- Meter per second
NaOH	- Sodium hydroxide
N m	- Newton meter
nm	- Nanometer
R_{et}	- Retention Ratio

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Soy milk is a soy product which is rich in protein and carbohydrates. It is extracted from ground soybeans to form a colloidal solution (Zhang *et al.,* 2005; Guo *et al.*, 1997). The major health benefit of soy products is their use as a dairy substitute in lactose intolerant patients. The enrichment of the protein fraction from soy products yields a high value protein concentrate with enormous economical potential in the health food industry. (Akoum *et al*., 2006).

The market for soybean protein products received a substantial boost in 1999 when the United States of America (USA) government allowed a health claim for food products containing at least 6.25 g of soy protein per serving can reduce the risk of heart disease. To meet the demand, a new generation of functional soy protein ingredients had to be created using innovative technology (Kumar *et al.,* 2004).

The use of membrane separations using ultrafiltration (UF) in the fractionation of soy protein from soy milk has generated considerable interest, recently. Compared to traditional methods, UF gives higher yield and superior functional properties of the soy product, in addition to the benefits of the non-thermal and non-chemical nature of the UF process (Kumar *et al.,* 2004; Cheryan, 1998). Moreover, the recovery of active soybean trypsin inhibitor (STI) for medical purposes by UF has added significant economic exploitation of the soybean.

In optimizing the UF membrane process of soy protein from soy milk, membrane fouling is one of the most important factors that will affect the membrane performance (Furukawa *et al.,* 2008). Fouling or accumulation of materials on the membrane can be observed when the permeate flux in an UF process does not increased linearly with transmembrane pressure (TMP) beyond a certain point. The pressure range in which the permeate flux increases with increase in TMP is referred to as the 'pressure dependant region', a region where the membrane is at its optimum performance (Ghosh, 2003).

Therefore in this study, a range value of TMP was tested in order to identify the value of TMP that give a better performance to the UF membrane in fractionation of soy protein from soy milk.

1.2 Problem Statements

Conventional method of protein fractionation like chromatography and electrophoresis faced several problems in term of scale up and the expensive equipments (Ghosh, 2003). The interest in usage of UF process in protein fractionation has developed from past 2 decades, but this UF process is strongly influenced by operating parameters like TMP. The optimization of the process seems to be the only way to make the process perfect.

There are limited published papers discussed about the optimized condition in UF process under the effects of TMP, but none used soy milk as raw material. The only research that has been done by manipulating TMP to investigate the performance of membrane is only to the model beer provided by Thomassen *et al.* 2005.

Thus this research is important to know the range of TMP that can be used to allow the membrane to perform at its best, in order to fractionate soy protein from soy milk.

1.3 Objective

The main objective of this research is to investigate the performance of the ultrafiltration (UF) membrane; in term of permeate flux, concentration of protein transmitted and retention ratio, by manipulating transmembrane pressure (TMP) to fractionate soy protein from soy milk.

1.4 Scope of Study

The soy milk is gone through the UF process at various TMP values ranging from 5 to 25 psi. Other parameters like membrane pose size, pH and feed temperature were held constant. The permeate flux and retention ratio at any given TMP value were calculated, along with the measurement of protein concentration transmitted and retained on the membrane. The results from parameters being tested remarked the best performance a membrane can achieve at any given TMP value.

CHAPTER 2

LITERATURE REVIEW

2.1 Protein Bioseparation

The phenomenal development of the modern biotechnology has made protein bioseparation more important at present moment than any other time before. Protein bioseparation refers to the recovery, isolation, purification, and polishing of protein products (Ghosh, 2003). The growing industry, demands more and more protein products in absolute purify form.

However, there are some characteristics of protein products that should be understand before the purification of protein can be done:

a. These products present at very low concentrations in their respective biological feed streams

b. These products present, along with large number of impurities that have chemical and physical properties similar to those of target product. Hence, bioseparation has to be selective in nature

c. The quality requirements for these products are frequently demanding.

d. These products are thermolabile, and hence many bioseparation techniques are usually carried out at sub-ambient temperature

e. These products are sensitive to operating conditions (such as pH and salt concentrations) and also to chemical substances (such as surfactants and solvents). The biological products are susceptible to denature and other forms of degradation in extreme conditions (Ghosh, 2006).

Thus, it is important for protein bioseparation to combine high productivity with high selectivity of separation. Protein bioseparation also must be feasible at mild operating conditions.

2.2 Economic Aspects of Protein Bioseparation

It is widely recognized that protein bioseparation is technically and economically challenging. The successful commercialization of protein-based product is depended on protein bioseparation, as it often regarded as the critical limiting factor, which usually is a substantial fraction of the total cost of production for most products of biological origin. Table 2.1 shows the bioseparation cost as approximate proportion of cost production for certain protein based products.

Table 2.1: Cost of protein bioseparation (Ghosh, 2003)

Products	**Approximate relative price**	**Bioseparation cost as percent of total cost of production**
Food/additives	1	10-30
Nutraceuticals	2-10	30-50
Industrial enzymes	5-10	30-50
Diagnostic enzymes	50-100	50-70
Therapeutic enzymes	50-500	60-80

As clearly indicated by these figures, bioseparation cost is the major cost of total production cost. Thus, it is very important to develop cost-effective isolation and purification processes.

2.3 The Recovery, Isolation, Purification and Polishing Scheme

A Recovery, Isolation, Purification and Polishing, (RIPP) scheme is commonly used in bioseparation (Ghosh, 2006). Table 2.2 lists the categories of RIPP scheme with some of the most commonly used protein bioseparation techniques.

The strategy of this scheme involves use of low resolution techniques, for example precipitation, filtration, centrifugation, and crystallization first for recovery and isolation, followed by high-resolution techniques, for example affinity separation, chromatography, and electrophoresis for purification and polishing. The high-throughput, low-resolution techniques are first used to significantly reduce the volume and overall concentration of the material being processed. The partially purified products are then further processed by high-resolution low-throughput techniques to obtain pure and polished finished products.

However, this scheme also has its disadvantages which include high capital cost, high operational cost and also lower recovery of products. Development of membrane separation processes and any other new types of separation creates potential to avoid this conventional RIPP scheme.

Table 2.2: Protein bioseparation techniques (Ghosh, 2003)

High-productivity, low-resolution
Cell disruption
Precipitation
Centrifugation
Liquid-liquid extraction
Microfiltration
Ultrafiltration (UF)
Supercritical fluid extraction
High-resolution, low-productivity
Ultracentrifugation
Packed bed chromatography
Affinity separation
Electropheresis
Supercritical fluid chromatography
High-resolution, high-productivity
Fluidised bed chromatography
Ultrafiltration (UF)
Monolith column chromatography

Membrane processes give high throughput and can be fine-tuned or optimized to give very high selectivity. The use of these new techniques can significantly cut down the number of steps needed for bioseparation (Ghosh, 2006). Note that Ultrafiltration (UF) is listed in two categories since the resolution in an UF process depends very much on how it is operated.

2.4 Soybean and Soy Milk

Soybean (*Glycine max*) is a nutritional plant which being consumed world wide, especially in the Asia region. It is believed to contain high concentration of proteins (40–50 percent), lipids (20–30 percent) and carbohydrates (26–30 percent), with daily average consumption is 20 to 80 g among Asian (Hernández-Ledesma *et al.,* 2005)

Ground soybeans can be soaked and grinded with water to produce soy milk. As the popular beverage among Asian population, soy milk which is a turbid and colloidal solution, contains almost all of its components of the soy seeds like protein, lipid, and saccharides (Zhang *et al.,* 2005; Guo *et al.*, 1997). The milk is regarded as being nutritious and cholesterol-free health foods. It is an excellent economical dairy substitute in lactose intolerant patients. In addition, soy milk and soy related products are also used extensively in infant formulas (Akoum *et al*., 2006). Table 2.3 indicates the nutrition composition of soy milk.

Table 2.3: Nutrition composition of soy milk (Dunne, 1975)

	Soymilk		Soymilk
Measure	1 C	Total lipid, g	4.7
Weight, g	245	Total saturated, g	0.5
Calories	81	Total unsaturated, g	2.04
Protein, g	6.7	Total monosaturated, g	0.8
Carbohydrate, g	4.4	Cholesterol, mg	0
Fiber, g	3.2		
Vitamin A, IU	78	Tryptophan, g	0.11
Vitamin B1, mg	0.4	Threonine, g	0.28
Vitamin B2, mg	0.17	Isoleucine, g	0.35
Vitamin B6, mg	0.1	Leucine, g	0.6
Vitamin B12, mg	0		
Niacin, mg	0.36	Lycine, g	0.44
Pantothenic acid, mg	0.12	Methionine, g	0.1
Folic acid, mg	3.7	Cystine, g	0.1
Vitamin C, mg	0	Phenylalanine, g	0.37
Vitamin E, IU	0.04	Tyrosine, g	0.27
Calcium, mg	9.8	Valine, g	0.345
Copper, mg	0.3	Arginine, g	0.5
Iron, mg	1.4	Histidine, g	0.17
Magnesium, mg	47	Alanine, g	0.3
Manganese, mg	0.42	Aspartic acid, g	0.84
Phosphorus, mg	120	Glutamic acid, g	1.35
Potassium, mg	346	Glycine, g	0.3
Selenium, mg	3.2	Proline, g	0.4
Sodium, mg	29	Serine, g	0.35
Zinc, mg	0.56		

The effects of soybean products on health have gained lot of interests in recent decades. On 26 October 1999, The United States Food and Drug Administration (FDA) authorized the Soy Protein Health Claim stating that 6.25 g of soy protein a day may reduce the risk of heart disease. Due to this health claim, the market is very responsive, that later allow the soybean foods continue to penetrate rapidly into western cultures and diets (Zhang *et al.,* 2005; Fukushima, 2001; Hermansson, 1978). Some other studies also stated that its consumption may alleviate menopausal symptoms (Hernández-Ledesma *et al.,* 2005; Messina, 2000), and reduce the risk of osteoporosis (Hernández-Ledesma *et al.,* 2005; Shetty *et al.,* 2004; Barnes *et al.,* 1991).

A study by Fournier *et al.,* 1998 also demonstrated an inverse association between diets containing high amounts of soybean products and low cancer incidence and mortality rates, particularly breast, colon and prostate cancer. Although the specific components that are responsible for this chemopreventive activity remain to be identified, isoflavones isolated from soybeans have been extensively studied (Hernández-Ledesma *et al.,* 2005; Shetty *et al.,* 2004). However, the capacity of soybean trypsin inhibitors (STI) for preventing cancer and other age-related disorders is recently receiving more attention (Hernández-Ledesma *et al.,* 2005; Omoni *et al.,* 2005).

2.5 Soybean Trypsin Inhibitors (STI)

Soybean trypsin inhibitor (STI) is the most prominent antinutritional factors that present in raw soybean, which can cause serious problems in processing of soy products (Akoum *et al.,* 2006). The two main STI in soybean are the Kunitz and Bowman–Birk inhibitors. The Bowman–Birk inhibitors have molecular weights of around 8 kiloDalton (kDa) (Malaki *et al.,* 2008; Sessa *et al.,* 2001) and studies on Kunitz inhibitors founded that they have a molecular weight of 20 kDa (Malaki *et al.,* 2008; Kim *et al.,* 1985).

STI, along with hemagglutinins, phytoestrogens, allergens and the raffinose and stachyose oligosaccharides, can pose serious health risks if not removed or de-activated during the processing of raw soybeans (Akoum *et al.,* 2006; Salunkhe, 1991; Wolf,